“Water is the most widely used substance, raw material, or ingredient in the production, processing and formulation of compendial articles.” Chapter 1231 of the United States Pharmacopeia (USP) begins with these words in the article “Water for pharmaceutical purposes.”

In 1996, the USP developed a new method, proposed by the Water Quality Committee of the US PhRMA, in order to control and measure the quality of the water used for all pharmaceutical products wishing to enter the US market, regardless of where they are produced. This method, USP <645>, defines the suitability of Purified Water (PW) and Water for Injection (WFI) samples through conductivity readings. The raw materials used during the manufacture of pharmaceutical products have a strong impact on the quality of the final product. For this and safety reasons, all products, testing labs or quality control for pharmaceutical industry must comply with the USP requirements.

This article provides a detailed description of the USP <645> requirements, and a guidance on the instrumentation specifications and usage, along with tips and tricks on how to meet with the norm's specifications in a clear, easy and defined way.

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1. **Instrument specifications and operating parameters**

USP <645> outlines the requirements of the conductivity measuring system, including the conductivity meter and sensor. Those requirements have implications on the calibration and verification standards used. Several considerations are discussed in this section.

**a) Conductivity meter calibration**

In order to give a predicted instrument conductivity response, the conductivity meter needs to be calibrated. This process describes the calibration of sensor inputs on the meter by replacing the sensor with a service tool. This tool is typically a set of certified resistors or a decade box.

The meter calibration is achieved by using NIST-traceable resistors (or equivalent), which have an accuracy of ± 0.1% of the stated value. This accuracy level can also be achieved through adjustable resistance devices, like a Wheatstone bridge for instance.

USP requires that the accuracy of the resistors used for meter calibration be within ± 0.1%. The resistors used by METTLER TOLEDO (MT) service technicians are NIST traceable, and the real value of the resistor is known to a precision of ± 0.001%. When a meter calibration is performed, the true resistor value (not the nominal value) is used. The true value of the certified resistor may differ from the nominal value by up to 0.3%. Note that the uncertainty value is the critical component to USP compliance here, not the difference between nominal and real value.

In addition, USP requires a minimum resolution of 0.1 μS/cm and an instrument accuracy of ± 0.1 μS/cm at 1.3 μS/cm, without taking the accuracy of the sensor cell constant into account. All MT conductivity meters have a resolution of 0.01 μS/cm or better in that range. The general accuracy of the instrument’s sensor input is 0.5% - calculated to ± 0.07 μS/cm at 1.3 μS/cm - well within the limits of compliance with the norm.
b) Sensor calibration and verification of the conductivity cell constant

Sensor calibration is indispensable for a reliable conductivity analysis, and hence essential to ensure the reliability and quality of the manufactured products. Calibration could be defined as establishing the difference between what should be measured and what a sensor really measures. For a conductivity sensor, calibration means determining its cell constant, which is a factor that considers the geometrical characteristics of a conductivity sensor.

The conductivity cell constant can be defined either by the user through a calibration using a standard solution of known conductivity, or by the manufacturer, who provides the cell constant in a certificate. Such a certified cell constant can be entered directly into the instrument what makes an additional calibration obsolete. For low conductivities the usage of a sensor with a certified cell constant is strongly recommended. The equipment used, as well as environmental controls yield a cell constant with a more accurate value than a sensor which is calibrated in a lab by the end-user. In addition, the simple entry of the cell constant makes the process much easier for the user than an additional calibration.

USP requires that the value of this cell constant must be known with an accuracy of ± 2% of the stated value. Compliance with this standard is ensured via regular sensor verification readings. A simple measurement of a reference solution of known conductivity is enough to assess the accuracy. It is crucial that the temperature compensation is deactivated and this reading is compared to temperature-conductivity tables (Table 1 is shown as an example) provided on the bottle labels and sachets of the conductivity standards.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>18</th>
<th>20</th>
<th>23</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (µS/cm ± 1 %)</td>
<td>8.56</td>
<td>8.97</td>
<td>9.59</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Table 1. METTLER TOLEDO conductivity standard for 10 µS/cm at temperature from 18 to 25 °C.

The sensor used must be accurate in a very low conductivity range and thus has a low nominal cell constant. Usually its shaft material is made of stainless steel or titanium, so as to be able to measure low conductivity values.

MT recommends using the following combinations of conductivity sensors and instruments:
1. InLab 741, InLab 741-ISM or InLab Trace (digital sensor) sensors for SevenExcellence and SevenCompact instruments.
2. InLab 742 or InLab 742-ISM sensors for Seven2Go Pro and SevenGo Duo Pro instruments.

The above listed sensors are delivered with a certified cell constant. Digital and ISM sensors have this value stored in the sensor’s memory chip, which is transferred to the instrument automatically. For non-ISM versions of InLab 741 and InLab 742 the cell constant can be manually entered into the instruments.
c) Use of non-temperature compensated conductivity readings

Conductivity measurements are very temperature sensitive. In order to account for the impact of temperature variations on the conductivity value, many instruments apply a correction factor. This factor normalizes the conductivity value, mathematically adjusting the reading to a reference temperature (normally to 25 or 20 °C).

This normalization is typically achieved by using an algorithm and a temperature probe, which is usually incorporated in the conductivity sensor. The algorithm is embedded in the instrument’s system and calculates the conductivity at nominal temperature using the correction factor. The accuracy of this algorithm may change depending on the sensor (it can be linear or non-linear). In order to perform all measurements in the same way, regardless of the accuracy of the algorithm of the sensor used, the temperature correction must be disabled in the instrument's setup.

d) Conductivity standard solutions used for calibration and verification of the cell constant

A major USP requirement is that the cell constant must be known within ± 2%. As mentioned above, the cell constant is either known and certified, or the sensor must first be calibrated in a standard solution in order to determine its value. In a second step, the value of the cell constant needs to be verified for all sensors (certified and non-certified).

One very common error is to perform the calibration, in the case the user carries it out, with a very low conductivity standard solution. Standards below and including 10 µS/cm are not suitable for an accurate calibration due to their instability, introducing error into the solutions themselves. The conductivity value of these standards increases quickly due to the absorption of atmospheric carbon dioxide, which leads to erroneously high conductivity values. It is advisable to use standards with slightly higher conductivity values for calibration so as to avoid this problem. MT recommends calibrating the sensor using the 84 μS/cm standard solution.

Once the sensor is calibrated, a subsequent verification measurement of conductivity is performed. It is advisable to do the verification measurement in a low conductivity standard solution (e.g. 10 µS/cm) since the conductivity of samples like PW and WFI samples is within this range. The sensor should first be rinsed with deionized water, then with the standard. In order to avoid contact with air as much as possible, the reading should be taken with the sensor directly in the bottle or using a flow-cell. In both cases the solution has to be discarded afterwards. The reading has to be within ± 2% of the solution’s tabulated conductivity value for the measured temperature.
2. Procedure

In order to measure the quality of PW and WFI through conductivity readings, a three-stage procedure must be followed:

a) Stage 1

Firstly, a water temperature measurement and a non-temperature compensated conductivity reading are performed. USP <645> requires a precision of ± 2 °C in temperature measurement. The temperature probe input of the MT Seven Series pH instruments has a limit of error of ± 0.1 °C. Both temperature probes that are built into MT InLab® conductivity electrodes as well as the external ones (separate InLab® temperature probes) have a tolerance of ± 1 °C or lower, perfectly in compliance with the norm.

The measured conductivity value is compared to a Temperature/Conductivity Limits table (Table 2). If the measured temperature value is not on the list, the next lower temperature must be taken in order to define the conductivity limit. Interpolation should not be performed.

If the measured conductivity value does not exceed the corresponding conductivity limit value on the table, the test is complete. If it is higher than the table value, then it is necessary to proceed to Stage 2.

It is recommended that the measurement is performed at-line using a sample bottle or beaker.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Conductivity Limit (µS/cm)</th>
<th>Temperature (°C)</th>
<th>Conductivity Limit (µS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6</td>
<td>55</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>60</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>0.9</td>
<td>65</td>
<td>2.5</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
<td>70</td>
<td>2.5</td>
</tr>
<tr>
<td>20</td>
<td>1.1</td>
<td>75</td>
<td>2.7</td>
</tr>
<tr>
<td>25</td>
<td>1.3</td>
<td>80</td>
<td>2.7</td>
</tr>
<tr>
<td>30</td>
<td>1.4</td>
<td>85</td>
<td>2.7</td>
</tr>
<tr>
<td>35</td>
<td>1.5</td>
<td>90</td>
<td>2.7</td>
</tr>
<tr>
<td>40</td>
<td>1.7</td>
<td>95</td>
<td>2.9</td>
</tr>
<tr>
<td>45</td>
<td>1.8</td>
<td>100</td>
<td>3.1</td>
</tr>
<tr>
<td>50</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Stage 1 – Conductivity Limits as a Function of Temperature.
b) Stage 2

As a first step, a sample of water with a volume of at least 100 mL is taken and transferred to a container. Before measuring the conductivity, the temperature of this water sample must be stable at 25 °C ± 1 °C, so consider a temperature adjustment and maintenance. Then, the sample is stirred, and hence is equilibrated with air.

No further conductivity readings should be taken until the conductivity value remains stable, keeping the same value over a period of 5 minutes (with a possible variation up to 0.1 µS/cm). At this time, the water sample is saturated with air due to the absorption of atmospheric carbon dioxide.

In case the new conductivity reading does not exceed 2.1 µS/cm, the test is successful. If the water test fails, Stage 3 needs to be performed so as to verify the water quality.

c) Stage 3

In order to adjust the ionic strength, saturated KCl is added to the previous sample (0.3 mL of KCl per 100 mL of water sample) and then pH is measured. This test should be done approximately 5 minutes after the end of Stage 2, and as before, the temperature needs to be kept relatively constant at 25 °C ± 1 °C throughout the development of the measurement.

If at that pH, the conductivity measured at Stage 2 does not exceed the conductivity limit in Table 3, the tested water sample complies with USP requirements.

<table>
<thead>
<tr>
<th>pH</th>
<th>Conductivity Limit (µS/cm)</th>
<th>pH</th>
<th>Conductivity Limit (µS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>4.7</td>
<td>6.1</td>
<td>2.4</td>
</tr>
<tr>
<td>5.1</td>
<td>4.1</td>
<td>6.2</td>
<td>2.5</td>
</tr>
<tr>
<td>5.2</td>
<td>3.6</td>
<td>6.3</td>
<td>2.4</td>
</tr>
<tr>
<td>5.3</td>
<td>3.3</td>
<td>6.4</td>
<td>2.3</td>
</tr>
<tr>
<td>5.4</td>
<td>3.0</td>
<td>6.5</td>
<td>2.2</td>
</tr>
<tr>
<td>5.5</td>
<td>2.8</td>
<td>6.6</td>
<td>2.1</td>
</tr>
<tr>
<td>5.6</td>
<td>2.6</td>
<td>6.7</td>
<td>2.6</td>
</tr>
<tr>
<td>5.7</td>
<td>2.5</td>
<td>6.8</td>
<td>3.1</td>
</tr>
<tr>
<td>5.8</td>
<td>2.4</td>
<td>6.9</td>
<td>3.8</td>
</tr>
<tr>
<td>5.9</td>
<td>2.4</td>
<td>7.0</td>
<td>4.6</td>
</tr>
<tr>
<td>6.0</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Stage 3 – Conductivity Limits as a Function of pH.

It is important to note that in Table 3 the pH range is 5 to 7. If the measured pH is outside of this range, or the measured conductivity is higher than the conductivity permissible at that pH value (on Table 3), then the water sample tested is not compliant with the norm.
3. Challenges and how to overcome them

METTLER TOLEDO’s conductivity meters, sensors and standard solutions help ensure compliance with USP <645> conductivity requirements with high accuracy, rendering the process fast and simple.

If many measurements are taken at the same time, it is relatively easy to make a mistake in table referencing. In order to avoid introducing any possible error, the SevenExcellence™ meter can deactivate the temperature compensation automatically in USP mode, compare results to the memory-integrated tables and indicate directly whether the sample meets the specifications or not. This is achieved by using a workflow method, which is a sequence of analysis steps with pre-set parameters. These workflow methods guide the user step by step on how to proceed with the instrument, and ensure users are following the proper SOP (Standard Operation Procedure).

SevenExcellence™ instrument has predefined methods in its memory. The following predefined SevenExcellence’s methods are available (in version 4.1), which cover a wide range of the steps required in USP 645 norm (Further information can be found in MT Application Brochure ‘21 METTLER TOLEDO Methods For SevenExcellence’):
1. Application M008 covers Stage 1 of the USP 645 conductivity regulation.
2. Application M030 to perform Stage 2 of USP 645 (if the previous method fails (M008), it is required to proceed to the Stage 2).
3. Application M031 for USP 645 Stage 3 (if method M030 does not meet the specifications of the norm).
4. Before starting Stage 3, MT recommends calibrating the pH electrode. M027 predefined method should be used for this purpose.

A workflow method can also be created for the calibration or verification. Additionally, in combination with LabX®, it is even possible to combine both calibration and verification methods in the same one, simplifying the process and assuring that no steps are forgotten.

Furthermore, if the market is regulated, as for the pharmaceutical industry, normally it is not enough just to verify that USP is fulfilled. SOP certificates need to be filled out, and the sensor cell constant certificate (if a certified sensor is used), the calibration and verification results must be saved. SevenExcellence™ instrument allows the transfer of data to a printer, to a computer, or a complete report can be created with LabX® software. All these reasons make SevenExcellence™ meter a highly recommended option for regulated markets, supporting compliance with strict regulations such as 21 CFR part 11.

An additional advantage of SevenExcellence™ is that both conductivity readings and the pH measurement required at stage 3 can be performed with the same instrument, equipped with the corresponding modules, avoiding the use of two instruments. MT recommends using the InLab® Pure Pro-ISM sensor for this pH measurement, which is a specific sensor designed for low-ionic strength samples like ultra-pure water. Its 1 mol/L KCl bridge electrolyte makes it possible to obtain more accurate results due to similar ionic strength between the electrolyte and the sample.

Last but not least, pH measurement specifications at stage 3 are not covered by USP <645> norm. A guidance on this procedure and instrumentation can be found in MT White Paper ‘Perform Your Next pH Measurement in compliance with USP <791>’.
4. Conclusion

Compliance with USP <645> norm is required for many applications related to the pharmaceutical industry. With a 3-step procedure, it is possible to introduce errors as tables are manually referenced, leading to inaccurate results or failed audits. Therefore, care must be taken.

A deciding factor in reducing measurement inaccuracies is choosing the right equipment. Picking the most adequate conductivity probe with a certified cell constant, along with the suitable conductivity standards for calibration and verification are key to an accurate and reliable measurement. Additionally, an appropriate instrument (especially equipped with predefined methods) aids in meeting with strict regulations, ensuring fast and accurate results in your testing. METTLER TOLEDO advises to take these factors into account and conduct your next conductivity measurement in accordance with these recommendations.

5. References


For more information www.mt.com